AD NUMBER

AD479136

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; FEB 1966. Other requests shall be referred to US Army Biological Center, Ft Detrick, Frederick, MD 21701.

AUTHORITY

BDRL D/A ltr, 28 Sep 1971

TECHNICAL MANUSCRIPT 277

INHIBITION OF PROTEIN SYNTHESIS BY SPERMINE IN GROWING CELLS OF STAPHYLOCOCCUS AUREUS

Mischa E. Friedman
Uriel Bachrach

FEBRUARY 1966

UNITED STATES ARMY
BIOLOGICAL CENTER
FORT DETRICK

DDC AVAILABILITY NOTICES

Qualified requestors may obtain copies of this publication from DDC.

Foreign announcement and dissemination of this publication by DDC is not authorized.

Release or announcement to the public is not authorized.

DISPOSITION INSTRUCTIONS

Destroy this publication when it is no longer needed. Do not return it to the originator.

The findings in this publication are not to be construed as an official Department of the Army position, unless so designated by other authorized documents.

U.S. ARMY BIOLOGICAL CENTER
Fort Detrick, Frederick, Maryland

TECHNICAL MANUSCRIPT 277

INHIBITION OF PROTEIN SYNTHESIS BY SPERMINE IN GROWING CELLS OF *STAPHYLOCOCCUS AUREUS*

Mischa E. Friedman

Uriel Bachrach

Medical Bacteriology Department
BIOLOGICAL SCIENCES LABORATORY

Project 1C014501B71A

February 1966

FOREWORD

The work reported in this manuscript was carried out under a Secretary of the Army Research and Study Fellowship awarded to Mischa E. Friedman. The work was done in collaboration with Professor Uriel Bachrach at the Department of Clinical Microbiology, Hadassah Medical School, Hebrew University, Jerusalem, Israel.

ACKNOWLEDGMENT

The excellent technical assistance of Miss Kochavit Mossafe is acknowledged.

ABSTRACT

Staphylococcus aureus SFL 9725 incorporated valine-C^{14} into cellular protein. This incorporation was inhibited by spermine when the pH of the culture was adjusted to 7.8 and the inhibition was antagonized partially by Mg^{++}. The incorporation of C^{14}-labeled leucine, phenylalanine, lysine, arginine, and possibly glutamic acid was inhibited to a much greater extent than that of alanine, glycine, or threonine. The uptake of spermine-C^{14} by S. aureus cells was rapid. More than 50% of the radioactivity resided in the soluble extract. The protein fraction of the soluble extract contained [illegible]% of the label, whereas the ribonucleic acid and deoxyribonucleic acid fractions contained little or no spermine-C^{14}.

I. INTRODUCTION

The polyamine spermine, $NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$, was found by Herbst and Snell[1] to be a growth factor for Hemophilus parainfluenzae. Mager[2] demonstrated that this cationic compound was protective toward bacterial spheroplasts and protoplasts. On the other hand, spermine inhibited the growth of Staphylococcus aureus[3] and Asnis and Rozanisky[4] later showed that spermine was bactericidal and that the adsorption of the polyamine by the cells decreased in the presence of high concentrations of hydrogen ion or other cations. Spermine was not detectable in S. aureus unless present in the growth medium. The same amount of polyamine was taken up per gram of cells at pH 8.0 or 6.0 and there was no evidence of its utilization. Spermine also acted as an antimutagen against induced mutation in S. aureus and other organisms[6] and Johnson and Bach[7] studying this effect in Escherichia coli found the incorporation of spermine to be greater in spheroplasts than in whole cells. The cell walls incorporated only 1% of the radioactive label and of that bound to the spheroplasts, only 50% was exchangeable. Studies of the activity of spermine at the molecular level[8-11] indicated a relationship between ribosomal material and spermine and consequently protein synthesis.

Bachrach and Persky[12] demonstrated the inhibition of protein synthesis by oxidized spermine by incorporating valine-C^{14} into cellular protein of E. coli. The effect of spermine on the incorporation of several C^{14}-amino acids by the whole cell system of S. aureus as well as studies on the incorporation and cellular distribution of labeled spermine are reported here.

II. MATERIALS AND METHODS

A. STOCK CULTURES AND MEDIA

S. aureus SFL 9725, a fresh isolate from human feces obtained from Dr. D. Sompolinsky, Assaf Harofe Government Hospital, Zerifin, Israel, was coagulase-negative, mannitol-negative, non-hemolytic and resistant to typing phages. According to Baird-Parker[13] this bacterium would not be classified as S. aureus, but as a member of Staphylococcus subgroup II. However, here the designation S. aureus SFL 9725 will be used. Stock cultures were maintained on Trypticase Soy Agar (BBL) supplemented with 0.001% thiamine and nicotinic acid, stored at 5 C, and transferred monthly. Broth media consisted of 1 or 2% Casitone (Difco), 0.2% glucose, 0.001% thiamine, and 0.001% nicotinic acid. The pH was 7.4. Inocula for growth studies consisted of cells harvested from 6-hr slants; denser inocula

from overnight slants were used in amino acid incorporation studies. All incubations were at 37 C with shaking. Growth was measured in the Klett-Summerson colorimeter with either the red, No. 66 or the green, No. 54 filter. E. coli B from the stock culture collection of the Department of Clinical Microbiology was cultured in the medium of Davis[14] supplemented with 0.1% peptone.

B. INCORPORATION OF C^{14}-AMINO ACIDS INTO BACTERIAL PROTEIN

The incorporation of C^{14}-amino acids into the protein of whole cells of S aureus or E. coli was examined by the method of Levinthal et al.[16] as follows: S. aureus was grown in 1% Casitone medium until the optical density (540 mμ) reached 0.3 to 0.35 (approximately 3 hours). After the pH of the culture was adjusted to 7.8 with N NaOH, labeled amino acid (2 μc/20 ml medium if L-isomer, 4 μc/20 ml if DL-) was added. Spermine was added simultaneously with the C^{14}-amino acid. Samples of 2.0 ml of the incubated mixture were removed at various times and pipetted into 2.0 ml of 10% trichloracetic acid (TCA) prepared in 1% Casamino Acids. Samples were well mixed, kept in ice for 30 min and centrifuged at 12,000 x g for 15 min. Each precipitate was suspended in 1.5 ml N NaOH, kept at room temperature for 20 min, then 6.0 ml of the 10% TCA solution were added and the mixture heated at 90 to 95 C for 30 min. After being cooled to room temperature, the suspensions were filtered through membrane filters* (0.45 μ pore size) previously soaked in 5% TCA in 1% Casamino Acids, washed twice with 10 ml quantities of the 5% TCA solution, and dried. The filters, mounted in planchets, were counted for radioactivity by a Nuclear Chicago thin window gas flow counter.

C. INCORPORATION AND DISTRIBUTION OF LABELED SPERMINE

When the density of the S. aureus culture reached 0.3 to 0.35, the pH was adjusted to 7.8 and spermine-C^{14}-tetrahydrochloride (0.25 μc/20 ml of medium) was added. At various time intervals 2.0 ml samples were diluted in 10.0 ml cold distilled water. The suspensions were filtered immediately through membrane filters, washed with additional aliquots of cold water, dried, mounted on planchets, and counted. Distribution studies were carried out with log phase cells that had been incubated 25 min in the presence of 0.0138 μc of spermine-C^{14} (1.13 μg/ml of medium). The cells were washed five times with cold 0.85% saline by centrifugation at 12,000 x g for 10 min at 4 C in the Sorvall RC-2 centrifuge, resuspended in saline, disrupted for 90 min in the Raytheon 10 kc sonic oscillator, and the soluble extract then treated in the manner described by Matthaei and Nirenberg[18] for the preparation of S-30 and S-100 supernatant solutions and 100,000 x g particulate matter. The cell debris was separated from whole cells by repeated centrifugation at 200 x g for 2 to 3 min at a time. All washes were with 0.85%

* Millipore Filter Corp., Bedford, Mass.

saline. Radioactivity of these materials was calculated per μg of ribonucleic acid (RNA). Following deproteinization by phenol extraction, the S-100 fluid was applied to a methylated albumin column[17] and the effluent fractions containing RNA and deoxyribonucleic acid (DNA) were located by ultraviolet absorption at 260 mμ in a Hitachi Perkin-Elmer spectrophotometer. The radioactivity of the protein and the collected effluents was measured in a Packard Tri-Carb liquid scintillation spectrometer with the samples dissolved in the scintillation liquid of Davidson and Feigelson.[18]

D. ANALYTICAL METHODS

Materials to be assayed colorimetrically for RNA were extracted first by the method of Schneider[19] and the extracts subjected to the orcinol test of Drury.[20]

E. RADIOCHEMICALS

L-Valine-1-C^{14}, specific activity 168 mc/mmole; DL-valine-1-C^{14}, 3.7 mc/mmole; DL-leucine-1-C^{14}, 36.6 mc/mmole; and uniformly labeled L-lysine-C^{14}, 7.5 mc/mmole; L-arginine-C^{14}, 7.7 mc/mmole; L-glutamic acid-C^{14}, 6.35 mc/mmole; and L-threonine-C^{14}, 5.47 mc/mmole were obtained from the Radiochemical Centre, Amersham, England. D-Alanine-1-C^{14}, 8.0 mc/mmole; glycine-1-C^{14}, 10.2 mc/mmole, and uniformly labeled L-alanine-C^{14}, 90 mc/mmole were purchased from Volk Radiochemical Co., Skokie, Illinois. L-Phenylalanine-1-C^{14}, 177 mc/mmole was obtained from Schwarz BioResearch, Inc., Orangeburg, N.Y.; and spermine-C^{14}-tetrahydrochloride, 4.25 mc/mmole, was purchased from New England Nuclear, Boston, Mass.

III. RESULTS

A. EFFECT OF SPERMINE ON THE GROWTH OF S. AUREUS

The growth of strain SFL 9725 was inhibited 50% by approximately 90 μg/ml of spermine and completely by approximately 120 μg/ml if the inhibitor was present at the time of inoculation (Fig. 1). The media for these tests were adjusted initially to pH 7.5. Stationary cultures were inhibited completely in the presence of approximately 50 μg/ml of spermine.

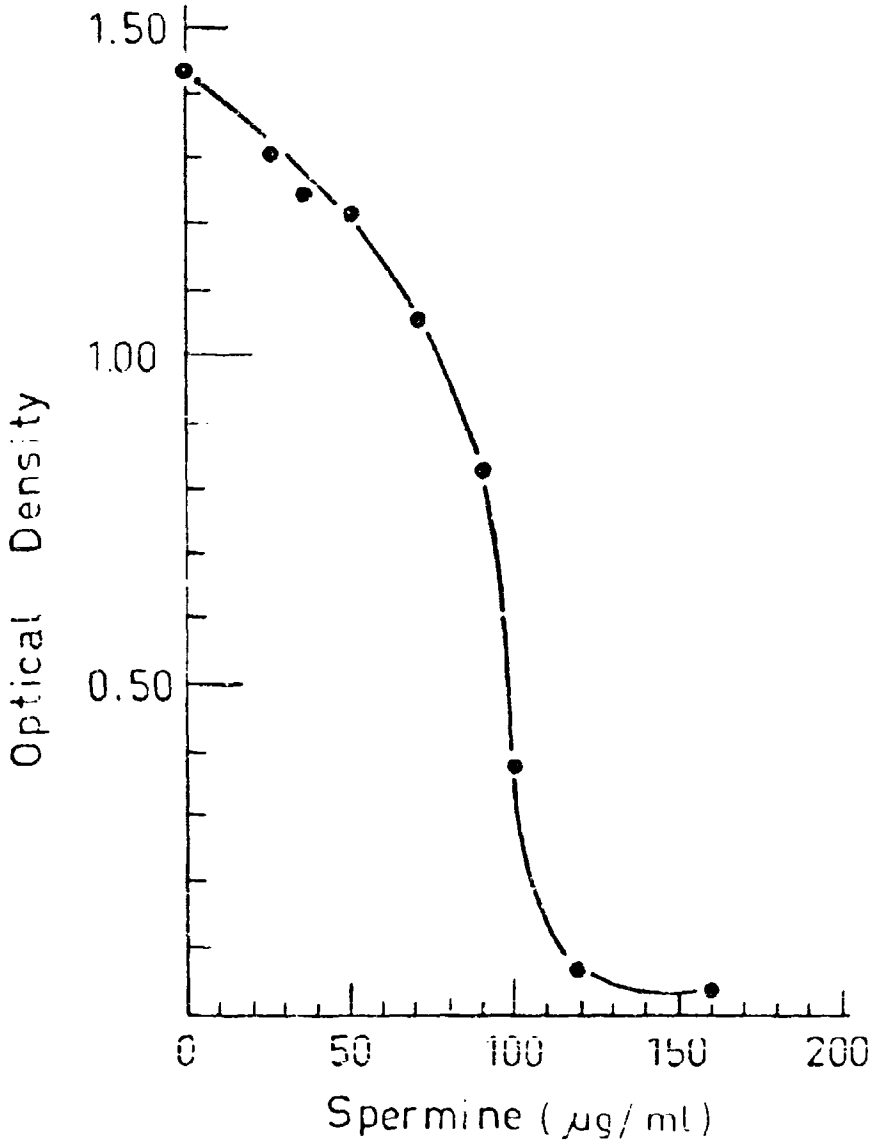

Figure 1. Effect of Spermine on Growth of S. aureus SFL 9725. Organisms were grown in 10-ml cultures in 150-ml Erlenmeyer flasks with shaking in a 37 C water bath.

B. INCORPORATION OF VALINE-C^{14}

Almost all of the counts from valine-C^{14} incorporated into growing log phase cells in 10 min were found in TCA-insoluble material indicating an incorporation into a high molecular weight polypeptide (Table 1). The addition of spermine to growing cells did not inhibit growth.

TABLE 1. INCORPORATION OF VALINE-C^{14} INTO GROWING LOG PHASE CELLS OF S. AUREUS SFL 9725[a]

Sample	Counts per Min per 2 ml Culture	Recovery, %
Control culture	642	
+ Spermine (90 µg/ml)	416	
+ Spermine (279 µg/ml)	212	
TCA-washed culture (A)	629	100
+ Spermine (B)	348	88
+ Spermine (C)	178	95
TCA wash A	28	
TCA wash B	20	
TCA wash C	24	

a. At density 0.3 (540 mµ), 14 µg DL-valine-C^{14} (4 µc) were added to 20 ml of culture adjusted to pH 7.8 with N NaOH simultaneously with the spermine. Following 10 min additional incubation with shaking in a 37 C water bath, samples were removed and treated according to Levinthal et al.[16] TCA-washed cultures were washed twice with 3 ml of 10% trichloracetic acid in 1% Casamino Acids by centrifugation before being given the Levinthal treatment. The first 5% TCA washes following the heating of the cells at 95 C were collected.

C. INHIBITION OF VALINE-C^{14} INCORPORATION BY SPERMINE AND EFFECT OF pH

The rapid incorporation of valine-C^{14} by log phase cells of S. aureus SFL 9725 was inhibited by spermine, but only if the pH of the culture was 7.6 or higher at the time of the addition of spermine (Fig. 2). Following a slight incorporation for the first 5 min, uptake ceased with 200 to 400 µg/ml of spermine. On the other hand, the incorporating system of E. coli was completely resistant to 209 µg/ml at pH 7.8 or 8.0.

D. EFFECT OF TIME OF ADDITION OF SPERMINE

When spermine (280 µg/ml) was added 5 min before the addition of valine-C^{14} to a culture adjusted to pH 7.8, the incorporation of radioactive material barely reached 50 cpm after 25 min (Fig. 3). This represents an approximate twofold increase in the effect of spermine over that observed if the inhibitor and valine-C^{14} were added together, and a tenfold increase if the spermine was added 5 min after the addition of the valine.

E. REVERSAL OF SPERMINE

Mg^{++} in the form of $MgCl_2$ caused a slight reversal of the effect of spermine, 2.3 mg/ml reducing the inhibition caused by 265 µg/ml spermine from 96 to 78%. No advantage was found by using an increased concentration of $MgCl_2$ or by replacing $MgCl_2$ with $MgSO_4$. Mn^{++} did not reverse the inhibition by spermine and although Ca^{++} reduced the inhibition of incorporation of counts approximately one-half, its use was disadvantageous, since it caused heavy precipitation in Casitone cultures at pH 7.8. Attempts to antagonize spermine with NH_4^+ ions were without consistent success.

F. EFFECT OF SPERMINE ON INCORPORATION OF OTHER C^{14}-AMINO ACIDS

Although log phase cells incorporated leucine-C^{14} and phenylalanine-C^{14} to a lesser degree than valine-C^{14}, spermine had the same inhibitory effect. On the other hand, incorporation of label from D-alanine, L-alanine, or glycine by S. aureus cells did not cease after an initial uptake but proceeded linearly at a slower rate than that of the control. An inhibition of 51 to 66% was observed after 20 min incubation (see curve for L-alanine, Fig. 4). The behavior of spermine toward the incorporation of label from lysine, arginine, and to a great extent glutamic acid was similar to that shown with valine (lable 2). The incorporation of threonine-C^{14}, however, was affected very little by spermine (Fig. 5).

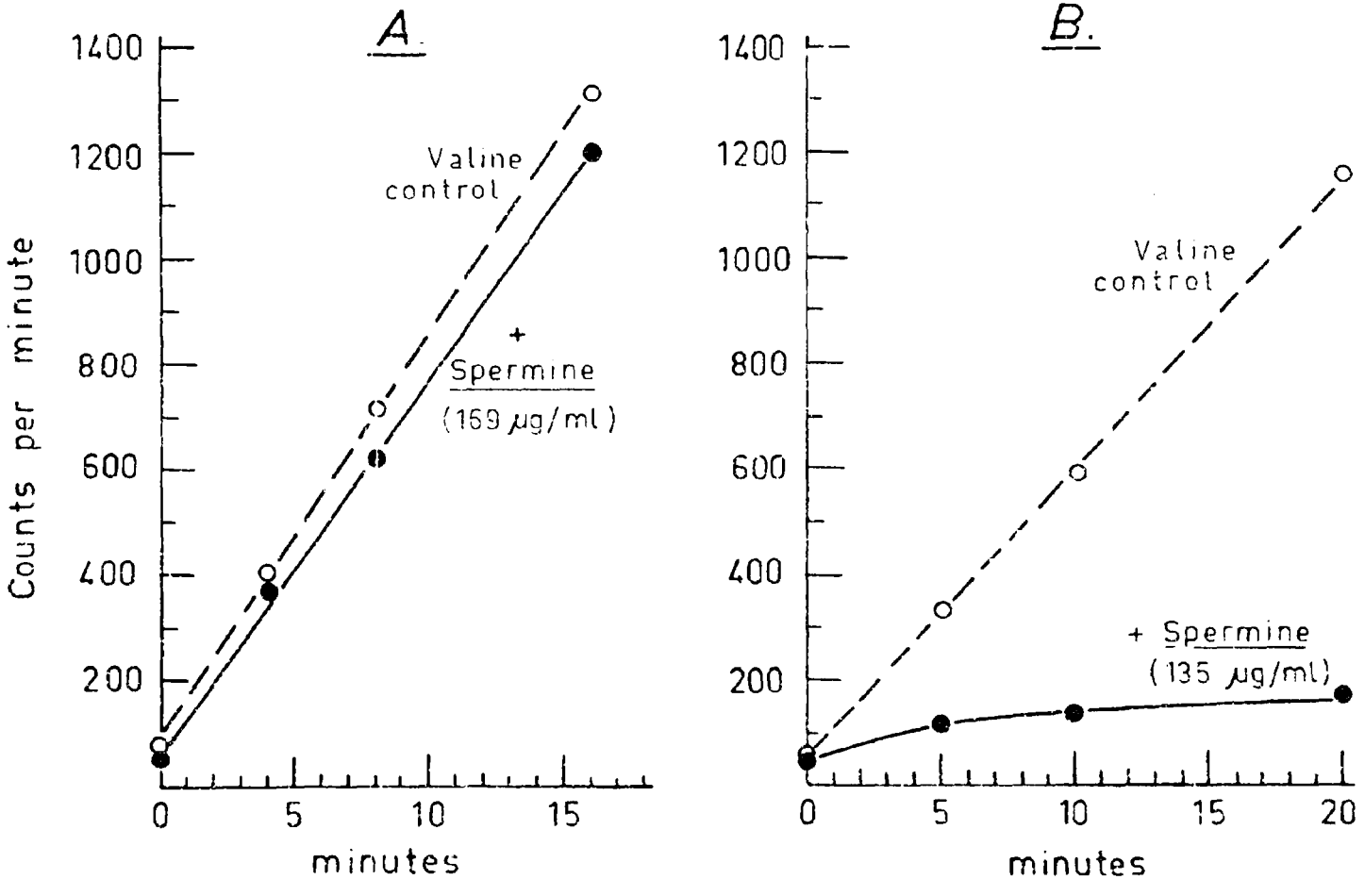

Figure 2. Effect of Spermine on Incorporation of Valine-C^{14} into Growing Log Phase Cells of S. aureus SFL 9725. (A) No pH adjustment. (B) pH of culture adjusted to pH 7.8 with concomitant addition of spermine and DL-valine-C^{14} (0.18 µc or 5.5 µg/ml). Organisms were grown in 20-ml culture in 150-ml Erlenmeyer flasks with shaking in a 37 C water bath.

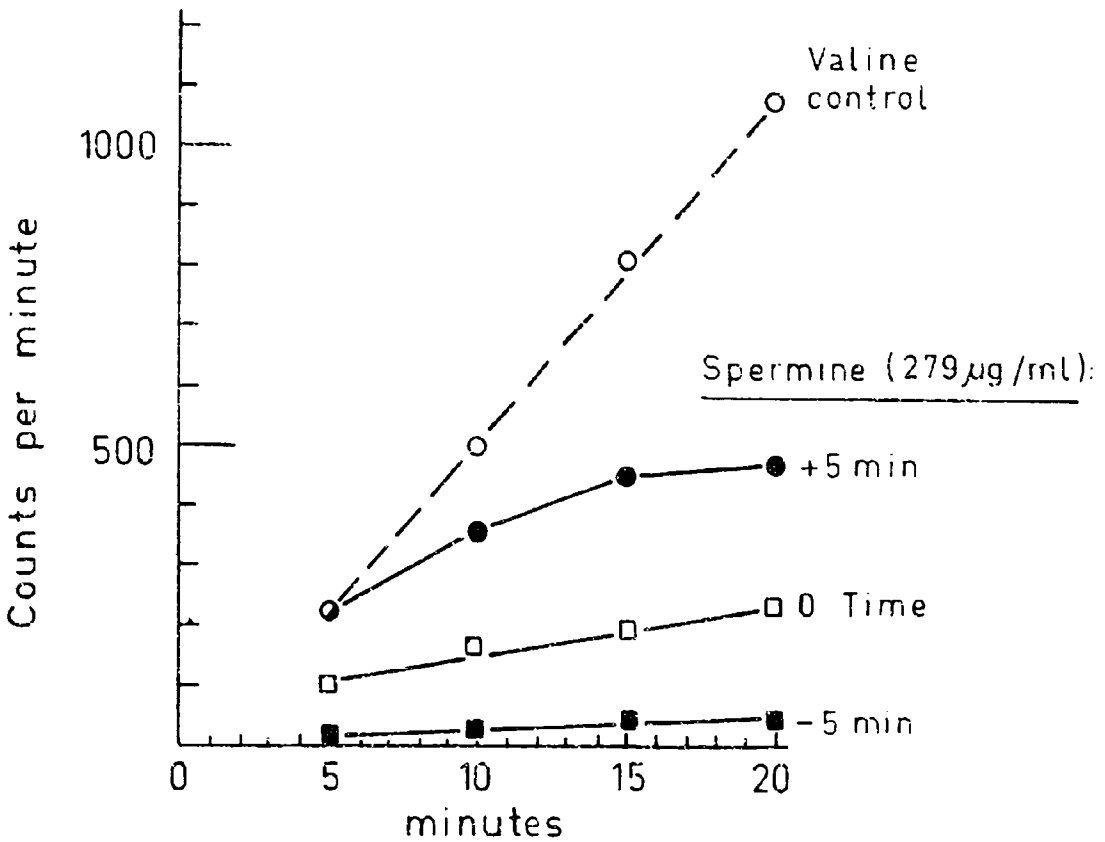

Figure 3. Effect of Time of Addition of Spermine on Incorporation of Valine-C^{14} into Growing Log Phase Cells of S. aureus SPL 9725. Spermine was added before, with, and after the addition of L-valine-C^{14} (0.1 µc or 0.07 µg/ml) to 20-ml culture at pH 7.8. The organisms were incubated with shaking in a 37 C water bath.

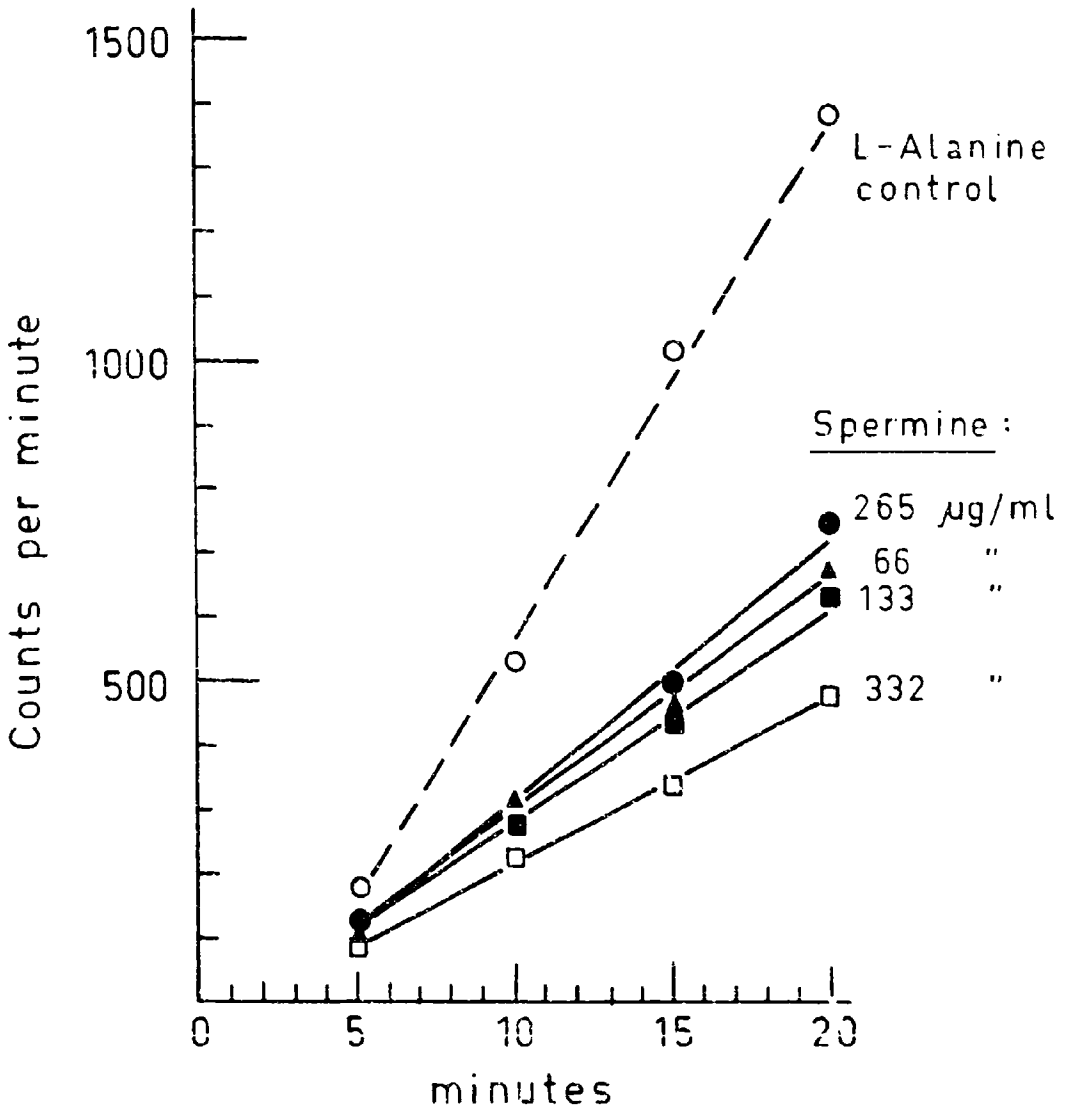

Figure 4. Effect of Spermine on Incorporation of L-alanine-C^{14} into Growing Log Phase Cells of S. aureus SFL 9725. The spermine and the L-alanine-C^{14} (0.1 µc or 0.1 µg/ml) were added together to 20 ml of culture at pH 7.8. Organisms were incubated with shaking in a 37 C water bath.

TABLE 2. EFFECT OF SPERMINE ON THE INCORPORATION OF C^{14}-AMINO ACIDS INTO GROWING LOG PHASE CELLS OF S. AUREUS SFL 9725

C^{14}-Amino Acid (μc/ml)	Spermine, μg/ml	Count per Min per 2 ml Culture[a]	Inhibition, %
DL-Valine (0.19)	0	981	
	135	172	83
DL-Leucine (0.19)	0	386	
	135	80	79
L-Phenylalanine (0.097)	0	328	
	135	53	84
L-Lysine (0.095)	0	362	
	133	108	70
	332	66	82
L-Arginine (0.095)	0	604	
	133	145	76
	332	65	89
L-Glutamic Acid (0.095)	0	891	
	133	252	72
	332	136	85

a. At density 0.3 (540 mμ) C^{14}-amino acid and spermine added together to 20 ml of culture adjusted to pH 7.8 with N NaOH. Following 20 min incubation with shaking in a 37 C water bath, 2.0 ml samples were removed and treated according to Levinthal et al.[18]

C. SPERMINE INCORPORATION AND CELLULAR DISTRIBUTION

The incorporation of spermine-C^{14} by multiplying log phase cells was rapid; the fastest rate occurred in the first 2 to 4 min (Fig. 6). The incorporation seemed to be antagonized by polylysine,* however, use of this compound for such experiments was disadvantageous, since it caused a precipitation even in sterile Casitone medium.

* Molecular Weight 3300, YEDA Research and Development Co., Rehoveth, Israel.

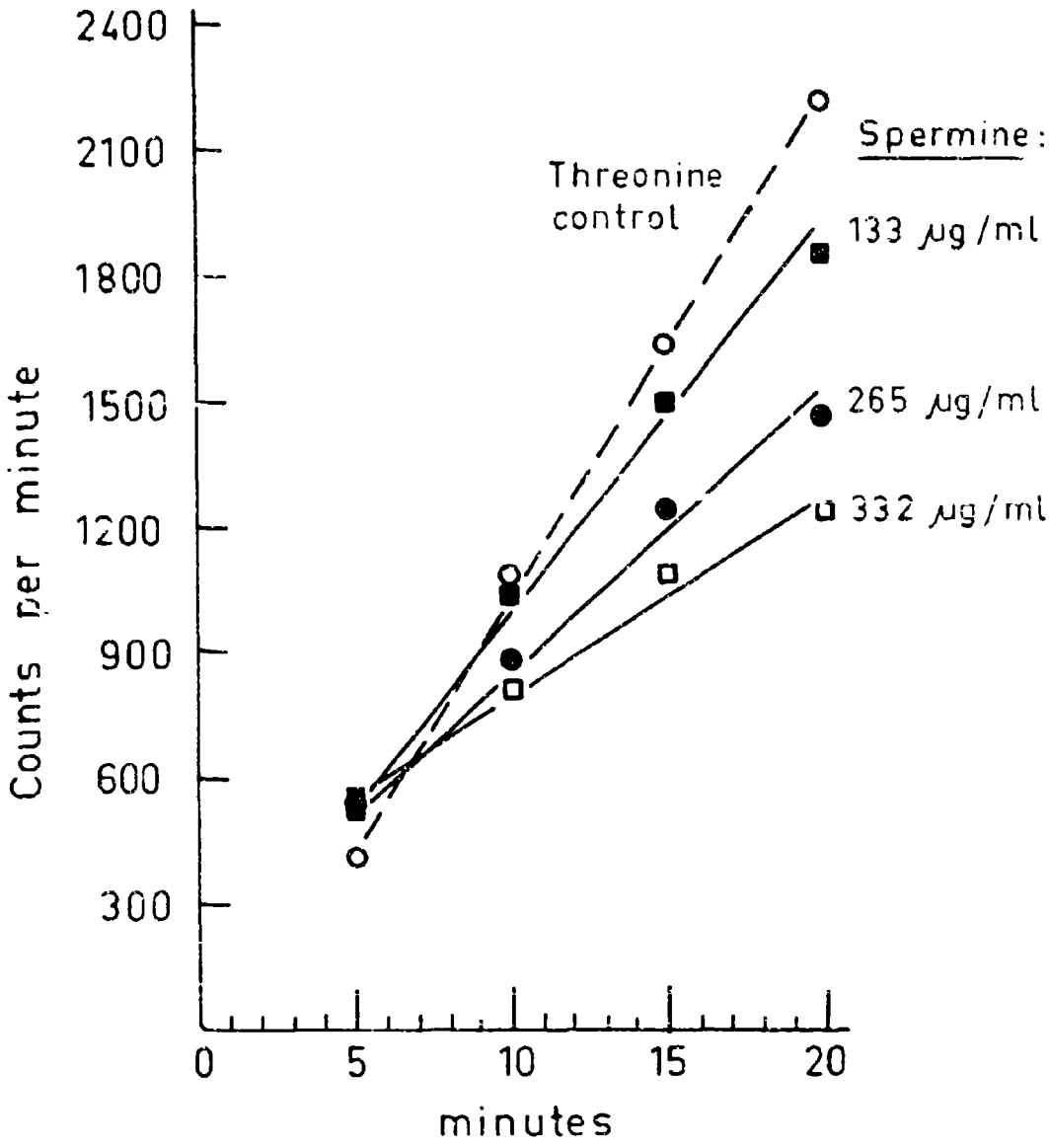

Figure 5. Effect of Spermine on Incorporation of Threonine-C^{14} into Growing Log Phase Cells of S. aureus SFL 9725. The spermine and the L-threonine-C^{14} (0.1 µc or 2.1 µg/ml) were added together to 20 ml of culture at pH 7.8. Organisms were incubated with shaking in a 37 C water bath.

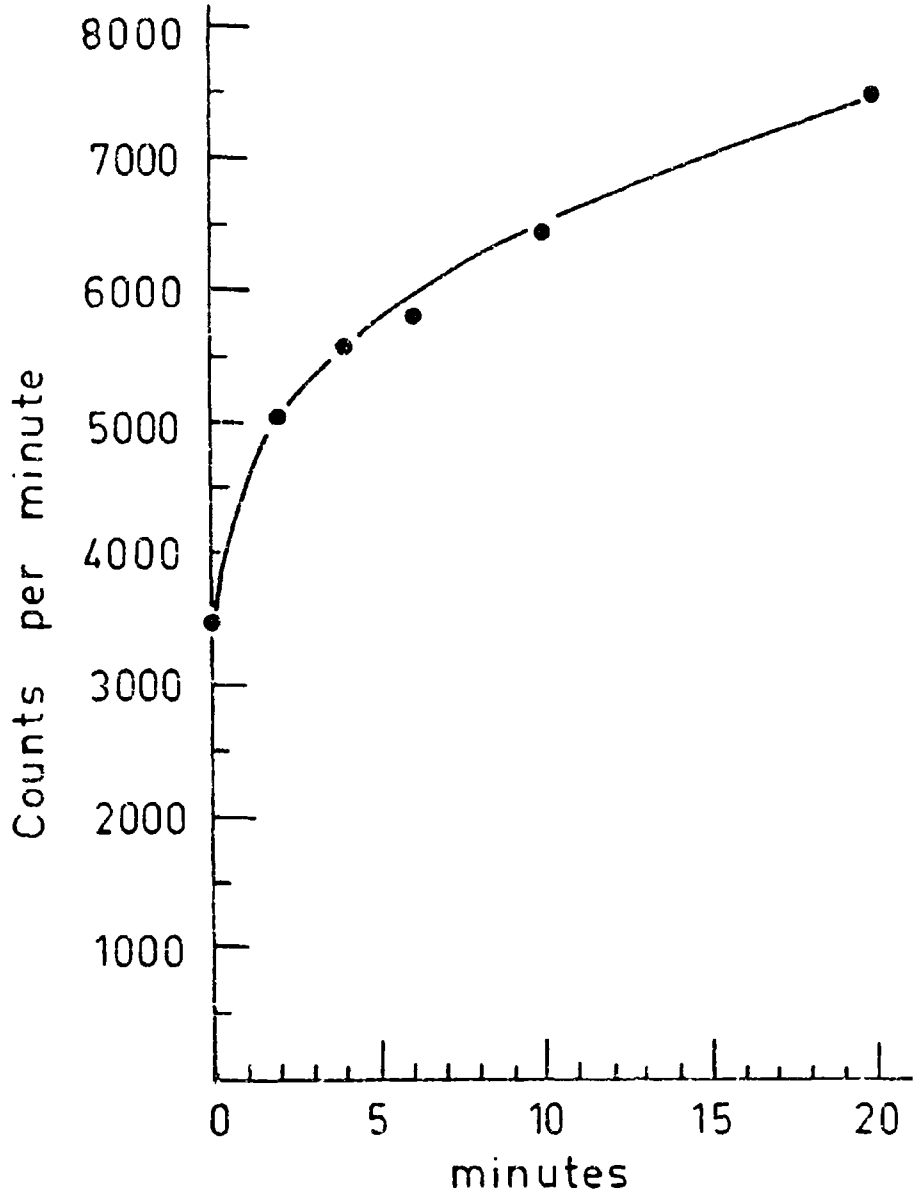

Figure 6. The Incorporation of Spermine-C^{14}-tetrahydrochloride (0.0125 μc or 1.0 μg/ml) into Growing Log Phase Cells of S. aureus SFL 9725. The culture, adjusted to pH 7.8, was incubated with shaking in a 37 C water bath.

When log phase cells were grown for 25 min in the presence of 0.0138 μc of spermine-C^{14} and then disrupted by sonic oscillation, 50% or more of the radioactivity recovered was found in the soluble cell extract. The 100,000 x g particulate matter contained a total of 3210 count/min and the S-100 supernatant fluid 134,200 count/min. If radioactivity was calculated as count/min per μg RNA, values of 15 were obtained for both the particulate and supernatant fractions. The count of the whole cells was 44 count/min per μg RNA. Following chromatography of the S-100 material through the methylated albumin column, the separated RNA and DNA fractions showed little radioactivity; 99% of the label was found in the protein fraction obtained by extraction with phenol.

IV. DISCUSSION

The growth inhibiting character of spermine towards S. aureus as shown by Rozansky et al.[3] was confirmed for strain SFL 9725. In addition, spermine was shown to inhibit the incorporation of valine-C^{14} by multiplying log phase S. aureus cells. The incorporation of counts leveled off following a short period during which a small amount of incorporation occurred. This was true also for labeled leucine, phenylalanine, arginine, lysine, and possibly glutamic acid, but not for L- or D-alanine, glycine, or threonine. With this latter group, no leveling off of incorporation was observed even in the presence of 332 μg/ml of spermine. These observations may reflect a possible effect spermine has on the permeation of amino acids into S. aureus. Also, the lack of complete inhibition of the incorporation of alanine and glycine may be explained as a failure of spermine to affect the incorporating system of these amino acids into cell wall mucopeptide.[21] Yet, lysine is known to be part of this glycopeptide but threonine has not been reported to be part of the staphylococcal cell wall peptide.

The effect of pH on spermine inhibitory activity and the role of Mg^{++} or polylysine in reversing this activity points to the cationic nature of the polyamine. The lack of complete reversal by Mg^{++} may have been due to the alkaline pH at which spermine activity is tested or to the possibility that the incorporated spermine was bound tightly to the polynucleotides of the cell.[22]

Growing S. aureus cells in the log phase incorporated spermine-C^{14} rapidly; cells in 1 ml of culture bound 0.14 μg of labeled spermine after 20 min. At least 50% of the bound spermine-C^{14} was in the particulate matter obtained after centrifugation at 100,000 x g. This finding is in agreement with the observation of Cohen and Lichtenstein[8] who found that spermine is tightly bound to bacterial ribosomes. Analysis of the 100,000 x g supernatant fraction on a methylated albumin column indicates

that spermine is not bound to soluble RNA or DNA. Yet redistribution of spermine-C^{14} during the phenol extraction has to be considered, although this treatment should not lead to a dissociation of the spermine-nucleic acid complexes.[23]

No definite explanation as to the mode of antibacterial action of spermine can be given as yet. However, the report of Mager et al.[10] and Friedman and Weinstein[11] strongly suggest that one activity may be similar to that of streptomycin in which genetic miscoding occurs resulting in the formation of incomplete or nonfunctional protein that could be lethal to the cell. It is remarkable that the code words of the amino acids[24] which were inhibited by spermine consist of either two adenylic or two uridylic acids. It is tempting to speculate that spermine is bound to these nucleotides or to the corresponding nucleotides in the anticodons of the s-RNA. The finding of Mandel[26] and Mahler and Mehrotra[86] that the effect of spermine on the melting temperature of DNA is a function of its adenine-thymine content also supports the view that adenylic and thymidylic acids (and possibly uridylic acid) are related to the biological activity of spermine. Obviously a more direct answer to the role of spermine in these reactions would have been obtained with a cell-free staphylococcal incorporating system utilizing synthetic messenger RNA. These studies will have to await the solution of the problems concerned with obtaining 70S ribosomes from Staphylococci.[27]

LITERATURE CITED

1. Herbst, E.J.; Snell, E. 1949. Putrescine and related compounds as growth factors for Hemophilus parainfluenzae 7901. J. Biol. Chem. 181:47-54.

2. Mager, J. 1959. The stabilizing effect of spermine and related polyamines on bacterial protoplasts. Biochim. Biophys. Acta 36: 529-531.

3. Rozansky, R.; Bachrach, U.; Grossowicz, N. 1954. Studies on the antibacterial action of spermine. J. Gen. Microbiol. 10:11-16.

4. Razin, S.; Rozansky, R. 1959. Mechanism of the antibacterial action of spermine. Arch. Biochem. Biophys. 81:36-54.

5. Rosenthal, S.M.; Dubin, D.T. 1962. Metabolism of polyamines by Staphylococcus. J. Bacteriol. 84:859-863.

6. Sevag, M.G.; Drabble, W.T. 1962. Prevention of the emergence of drug-resistant bacteria by polyamines. Biochem. Biophys. Res. Commun. 8:446-452.

7. Johnson, H.G.; Bach, M.K. 1965. Experiments on the site of binding of radioactive spermine in Escherichia coli. Bacteriol. Proc. p. 17.

8. Cohen, S.S.; Lichtenstein, J. 1960. Polyamines and ribosomal structure. J. Biol. Chem. 235:2112-2116.

9. Martin, R.G.; Ames, B.N. 1962. The effect of polyamines and of poly U size on phenylalanine incorporation. Proc. Nat. Acad. Sci. 48:2171-2178.

10. Mager, J.; Benedict, M.; Artman, M. 1962. A common site of action for polyamines and streptomycin. Biochim. Biophys. Acta 62:202-204.

11. Friedman, S.M.; Weinstein, I.B. 1964. Lack of fidelity in the translation of the synthetic polyribonucleotides. Proc. Nat. Acad. Sci. 52-988-996.

12. Bachrach, U.; Persky, S. 1964. Antibacterial action of oxidized spermine. J. Gen. Microbiol. 37:195-204.

13. Baird-Parker, A.C. 1965. The classification of staphylococci and micrococci from world-wide sources. J. Gen. Microbiol 38:363-387.

14. Davis, B.D. 1949. The isolation of biochemically deficient mutants of bacteria by means of penicillin. Proc. Nat. Acad. Sci. 35:1-10.

15. Levinthal, C.; Keynan, A.; Higa, A. 1962. Messenger RNA turnover and protein synthesis in B. subtilis inhibited by actinomycin D. Proc. Nat. Acad. Sci. 48:1631-1638.

16. Matthaei, H.; Nirenberg, M.W. 1961. The dependence of cell-free protein synthesis in E. coli upon RNA prepared from ribosomes. Biochem. Biophys. Res. Commun. 4:404-408.

17. Mandell, J.D.; Hershey, A.D. 1960. A fractionating column for analysis of nucleic acids. Anal. Biochem. 1:66-77.

18. Davidson, J.D.; Feigelson, P. 1957. Practical aspects of internal-sample liquid-scintillation counting. Int. J. Appl. Radiat. Isotop. 2:1-18.

19. Schneider, W.C. 1945. Phosphorus compounds in animal tissues: I. Extraction and estimation of deoxypentose nucleic acid and of pentose nucleic acid. J. Biol. Chem. 161:293-303.

20. Drury, H.F. 1948. Identification and estimation of pentoses in the presence of glucose. Arch. Biochem. 19:455-466.

21. Strominger, J.L. 1962. Biosynthesis of bacterial cell walls, p. 413-470. In I.C. Gunsalus and R.Y. Stanier (ed.) The bacteria, Vol. III. Academic Press, Inc., New York.

22. Felsenfeld, G.; Huang, S. 1960. The interaction of polynucleotides with metal ions, amino acids, and polyamines. Biochim. Biophys. Acta 37:425-433.

23. Shepherd, G.R.; Hopkins, P.A. 1963. The distribution of certain proteins, polypeptides, and polyamines in a aqueous-phenol system. Biochem. Biophys. Res. Commun. 10:103-108.

24. Nirenberg, M.; Leder, P.; Bernfield, M.; Brimacombe, R.; Trupin, J.; Rottman, F.; O'Neal, C. 1965. RNA codewords and protein synthesis: VII. On the general nature of the RNA code. Proc. Nat. Acad. Sci. 53:1161-1168.

25. Mandel, M. 1962. The interaction of spermine and native deoxyribonucleic acid. J. Mol. Biol. 5:435-441.

26. Mahler, H.R.; Mehrotra, B.D. 1962. Dependence of deoxyribonucleic-amine interactions on deoxyribonucleic acid composition. Biochim. Biophys. Acta 55:252-254.

27. Young, R.J.; Barker, G.R. 1964. The ribosomes of Staphylococcus aureus (Strain Duncan). Biochem. J. 91:22-24C.

Unclassified
Security Classification

DOCUMENT CONTROL DATA - R&D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1 ORIGINATING ACTIVITY (Corporate author) U.S. Army Biological Center Fort Detrick, Frederick, Maryland, 21701	2a REPORT SECURITY CLASSIFICATION Unclassified 2b GROUP

3 REPORT TITLE

INHIBITION OF PROTEIN SYNTHESIS BY SPERMINE IN GROWING CELLS OF STAPHYLOCOCCUS AUREUS

4 DESCRIPTIVE NOTES (Type of report and inclusive dates)

5 AUTHOR(S) (Last name, first name, initial)

Friedman, Mischa E.
Lachrach, Uriel NMI

6 REPORT DATE February 1966	7a TOTAL NO OF PAGES 22	7b NO OF REFS 27
8a CONTRACT OR GRANT NO. b PROJECT NO. 1C014501B71A c. d.	9a ORIGINATOR'S REPORT NUMBER(S) Technical Manuscript 277 9b OTHER REPORT NO(S) (Any other numbers that may be assigned this report)	

10 AVAILABILITY/LIMITATION NOTICES

Qualified requestors may obtain copies of this publication from DDC.
Foreign announcement and dissemination of this publication by DDC is not authorized.
Release or announcement to the public is not authorized.

11 SUPPLEMENTARY NOTES	12 SPONSORING MILITARY ACTIVITY U.S. Army Biological Center Fort Detrick, Frederick, Maryland, 21701

13 ABSTRACT

Staphylococcus aureus SFL 9725 incorporated valine-C^{14} into cellular protein. This incorporation was inhibited by spermine when the pH of the culture was adjusted to 7.8 and the inhibition was antagonized partially by Mg^{++}. The incorporation of C^{14}-labeled leucine, phenylalanine, lysine, arginine, and possibly glutamic acid was inhibited to a much greater extent than that of alanine, glycine, or threonine. The uptake of spermine-C^{14} by S. aureus cells was rapid. More than 50% of the radioactivity resided in the soluble extract. The protein fraction of the soluble extract contained 99% of the label, whereas the ribonucleic acid and deoxyribonucleic acid fractions contained little or no spermine-C^{14}.

DD FORM 1 JAN 64 1473

Unclassified
Security Classification